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The Role of Microorganisms and Invertebrate Soil Animals in Degradation of Bark: A Documentation by Scanning Electron Microscopy[1])

(Die Rolle von Mikroorganismen und wirbellosen Bodentieren beim Abbau von Baumrinde: Eine Dokumentation mit Hilfe der Rasterelektronenmikroskopie)

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With 14 figures

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Summary

Scanning electron microscopy is used for a documentation of the microecology of the organisms involved in the decomposition of bark. Above all the degradation of cell walls by bacteria and fungi is illustrated. The dense colonization of the substrate by microorganisms documents the diversity of the species involved in degradation. The presence of invertebrate soil animals indicates that their role in the process of decomposition should not be underestimated.

Zusammenfassung

Mit Hilfe der Rasterelektronenmikroskopie wird ein Einblick in die Mikroökologie der an der Kompostierung von Baumrinde beteiligten Organismen vermittelt. Dabei wird insbesondere der Abbau der Zellwände durch Bakterien und Pilze veranschaulicht. Die zum Teil dichte Besiedelung des Substrats durch Mikroorganismen dokumentiert die große Vielfalt der am Abbau beteiligten Arten. Die Präsenz von wirbellosen Bodentieren deutet darauf hin, daß deren Aktivität beim Abbau nicht unterschätzt werden sollte.

I. Introduction

In wood processing industries bark is often obtained as a byproduct which can be successfully utilized by composting. The aim of composting is to yield, by semi-controlled and aerobic activity, a substrate which is compatible with plants (KRAPFENBAUER, 1977; KAWADA, 1978). Since the nitrogen content of raw bark is relatively low, bark chips have to be mixed with nitrogen-rich material in order to allow efficient growth of decomposing microorganisms. For this reason fertilizers like ammonium sulfate or natural nitrogen sources such as waste or sludge are added to raw bark (OSTALSKI, 1976; JAHN, 1974). During the process of composting which can last up to several months the microorganisms break open and decompose the cellular structures of the bark. At the same time very resistant components like lignines or suberine are also degraded (LEVI and PRESTON, 1965; WILHELM, 1975; HÜTTERMANN et al., 1980). Composted bark can be compared with a high-quality soil because of its adsorption capacity, its porosity and water uptake properties (ALESTALO and KOISTINEN, 1975).

Scanning electron microscopy appears to be a good approach in order to obtain a direct insight into the event of degradation, since bark remains a relatively solid substrate throughout the whole process of decomposition.

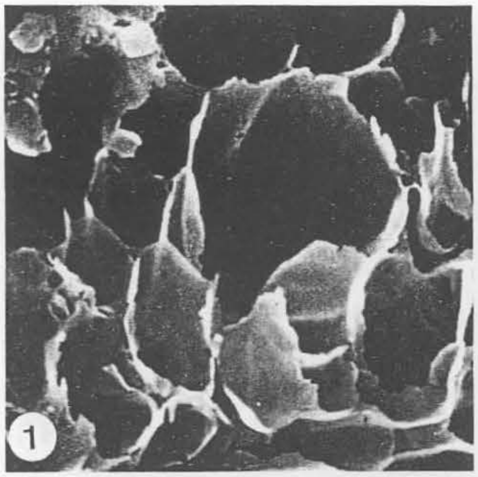
II. Material and methods

The bark was obtained from the processing of a sawmill belonging to the "Bundesforste" in Kramsach (Tirol). It originated mainly from spruce stem and to a lesser extent from deciduous wood. Composting was done by building up heaps in an area belonging to a field laboratory near Pill (Tirol) which was managed by the "Arbeitsgemeinschaft für Abfallwirtschaft – Tirol". The compost heaps contained 2600 kg of bark which amounted to a volume of 11 m^3. The heaps had a rectangular basis of 4 × 4 m and a height of 2 m. To the raw material fertilizers such as ammonium sulfate, urea, nitro-chalk and lime nitrogen were added in amounts of 0.5 to 1 kg per 100 kg of bark. One single heap without any nutrient addition was used as a control.

The samples for scanning electron microscopy were taken after a composting period of about 5 months. It was ascertained by chemical analyses that at that point the degradation had made good progress (DALLINGER and TSCHÖRNER, 1985). Samples were taken 20 and 70 cm below the top of the compost heaps, their size ranging from 3 × 3 × 1 to 5 × 5 × 2 cm. Samples from the middle of the experimental heaps on macroscopic oberservation appeared to be strongly overgrown with fungal *hyphae*, whereas those from the control heap seemed to be colonized to a lesser extent. Bark chips were wrapped in aluminium foil and stored overnight in a refrigerator at 4° C. The following day small cubic pieces about 0.5 cm in length were fixed in 6.25 % glutaraldehyde and 1 % osmium tetroxide and washed with phosphate buffer at pH 7.4. The samples were dehydrated in ethanol followed by critical point treatment. They were sputtered with gold and oberserved on a JEOL SU3 scanning electron microscope.

III. Results and discussion

Fig. 1 shows a relatively intact tissue from fresh bark. The cell walls appear smooth and show only few traces of microbial colonization. The holes in the cell walls are likely to be pit connections. Figs. 2–4 demonstrate the appearance of walls of bark cells after progressive degradation. The surfaces are very rough and perforated. The pit connections appear to be enlarged by microbial enzymatic activity and are strongly corroded. There are also pit connections near which large areas of totally degraded cell walls can be observed. It has been shown by WILHELM (1975) that fungal *hyphae* can infect sclereid cells by perforation of pit connections. Many of small holes seem to be due to direct microbial attack. The figures presented here give an insight into the degradation process and suggest that the break-up of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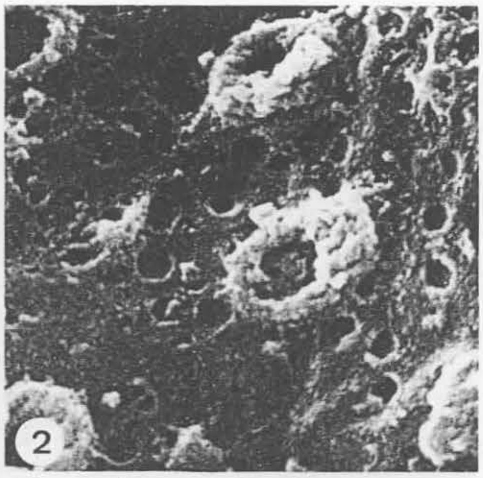
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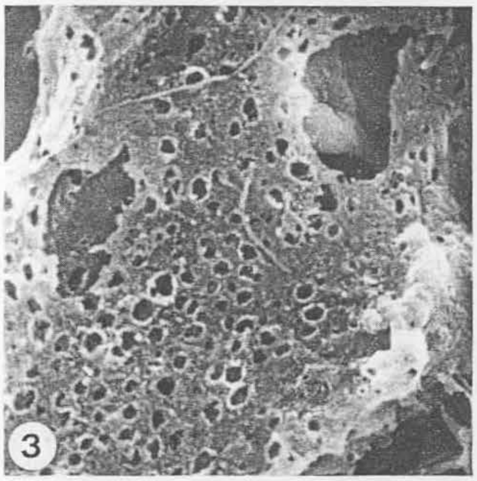
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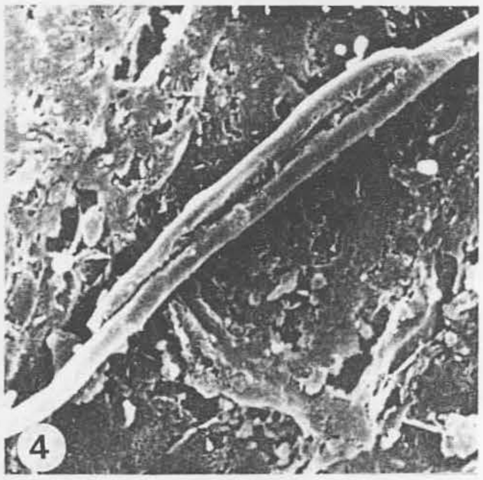
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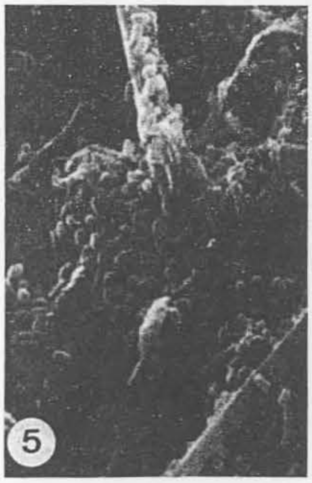
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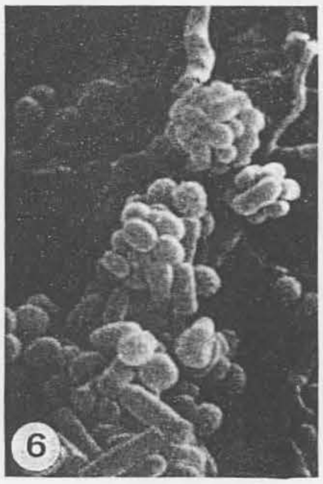
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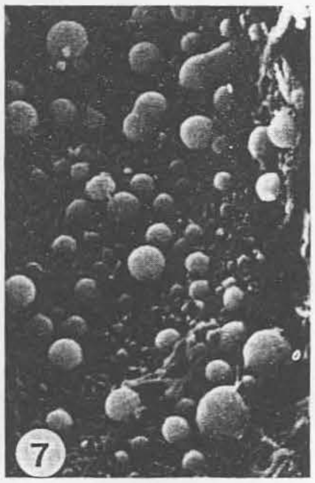
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cell walls may lead to a higher capacity of water uptake. The increase of internal surfaces may result in an increased adsorption capacity for minerals and trace elements (ZÖTTL, 1977).

Figs. 5–10 give an impression of the diversity of species of microorganisms involved in degradation. On the basis of the pictures obtained it was not possible, however, to identify species. Nevertheless, most of illustrated microorganisms can be identified to the level of different taxonomic groups. Figs. 5 and 6 give a view of the dense colonization of substrate by bacteria. They play a dominant role in decomposition and during the thermophilic phase of composting may amount to 100 % of total microorganisms (BÄGSTAM, 1978).

Figs. 7–10 show mainly fungi. As with bacteria, correct identification based on the pictures was possible only in a few cases (ALEXANDER, 1977). Among the organisms observed there are mainly imperfect fungi and yeasts. Basidiomycetal hyphae with clemp connections have also been found in a few cases.

It appears that besides bacteria and fungi a number of invertebrate soil animals like protozoans, nematodes, mites, and insects play an important role in bark degradation (DINDAL, 1978). It is well known, for example, that many species of mites and collemboles feed on bacteria and fungal hyphae, or even on decaying wood (SCHALLER, 1968). In compost bark heaps these arthropods are likely to encounter suitable conditions and one has to ask whether and to which extent they may delay the growth of decomposing microorganisms. Undoubtedly the invertrebrate soil animals are of great importance in the ecology of degradation. Their activity in bark composting is documented by figs. 11–14.

So even if the pictures shown in this work do not present any information on the mass turnover during composting, they bear substantial information about the complexity of biotic factors involved in the degradation of bark.

Figure 1: Intact bark tissue (probably parenchymatic and pitted cells) with smooth cell walls and pit connections. Scale: 1300:1.

Figure 2: View into a tracheid cell of deciduous wood with enzymatically enlarged pit connections and roughly perforated cell walls. Scale: 3800:1.

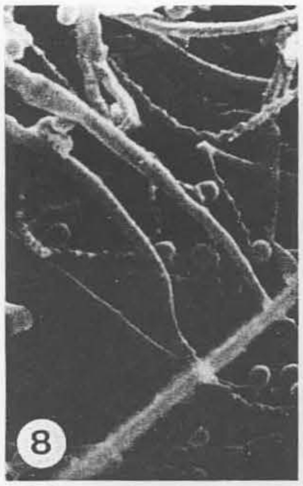
Figure 3: Cell wall with pit connections and extended areas of degradation. In the centre of the picture actinomycetes perforating the cell wall can be seen. Scale: 2850:1.

Figure 4: Cell wall with irregular fractures and holes which apparently represent traces of degradation. The cell wall is overgrown with a branched fungal hypha. Scale: 3100:1.

Figure 5: Dense Colonization of bark by bacillar bacteria and by structures resembling hyphae. Scale: 1300:1.

Figure 6: Aggregation of spherical, bacillar, and filamentous bacteria near cells with ornamented surface resembling conidiospores of Penicillium *and/or* Aspergillus *spp or bacteria like those observed in humus samples by* OLAH *et al. (1978) using scanning electron microscopy. Scale: 4500:1.*

Figure 7: View into a parenchymatic cell with dense colonization by differently sized and irregularly shaped organisms which in spite of their small size resemble yeasts. Scale: 4100:1.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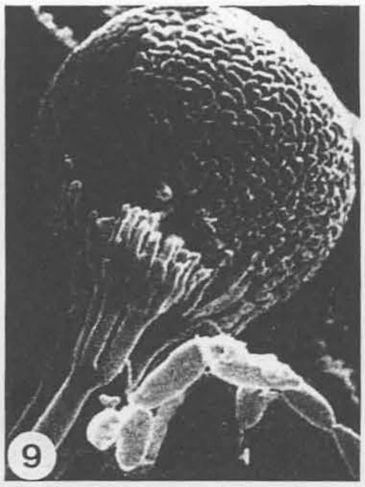
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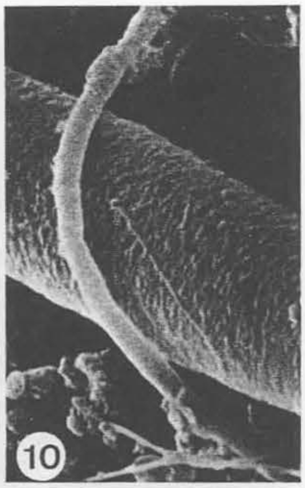
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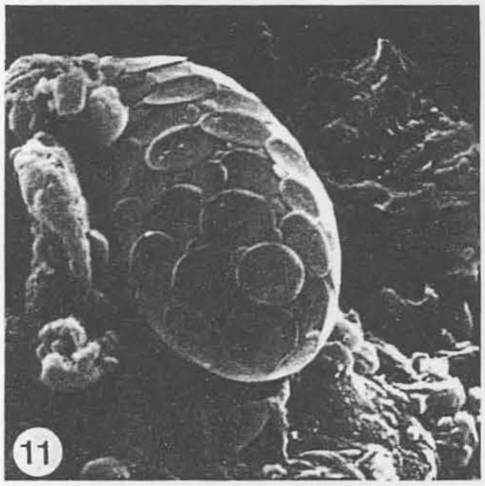
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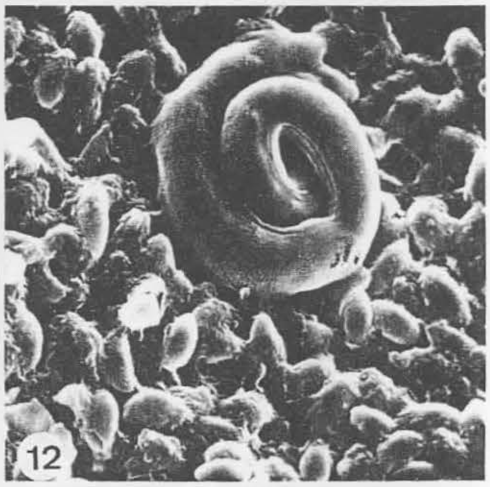
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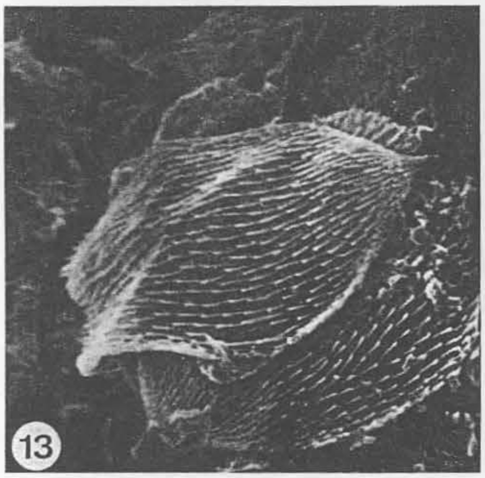
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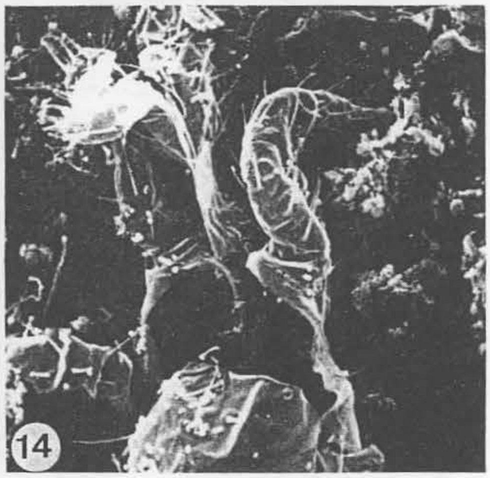
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Figure 8: Imperfect fungus with conidia. Scale: 3100:1.

Figure 9: Conidiophore of a fungus of the Penicillium-*type. It is covered with three-lobed conidiospores. Their shape and small size with a diameter of ca. 1.5 µm are very remarkable and so far unknown to us. Scale: 3100:1.*

Figure 10: Fungal hyphae with thin filamentous bacteria. Scale: 2850:1.

Figure 11: Shell of a Thecamoeba composed of circular calcareous plates. Scale: 2850:1.

Figure 12: Coiled nematode in the middle of its spawn. Scale: 900:1.

Figure 13: Squama which may have originated from a collembole. Scale: 2850:1.

Figure 14: Exuvia of a mite. Scale: 900:1.

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